



Exploiting senescence for the treatment of cancer

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Abstract | Senescence is a cellular response to a variety of stress signals that is characterized by a stable withdrawal from the cell cycle and major changes in cell morphology and physiology. While most research on senescence has been performed on non-cancer cells, it is evident that cancer cells can also mount a senescence response. In this Review, we discuss how senescence can be induced in cancer cells. We describe the distinctive features of senescent cancer cells and how these changes in cellular physiology might be exploited for the selective eradication of these cells (senolysis). We discuss activation of the host immune system as a particularly attractive way to clear senescent cancer cells. Finally, we consider the challenges and opportunities provided by a 'one-two punch' sequential treatment of cancer with pro-senescence therapy followed by senolytic therapy.

Senescence-associated β -galactosidase (SA- β -gal). Acidic β -galactosidase activity that occurs in senescent cells; used as a senescence biomarker.

Cells progressively accumulate damage during their lifetime due to a multitude of endogenous and exogenous stressors. Although an array of repair mechanisms exists, the damage can sometimes be persistent. Replicative cells also require defence mechanisms to reduce the impact of these persistent stresses on tissue degeneration and to restrict cancer development. One of these defence mechanisms involves switching to a stable non-proliferative, metabolically active survival state named cellular senescence. Cellular senescence can be induced by a variety of stress and damage signals. The phenomenon was first described in 1961 by Hayflick and Moorhead¹, who observed that replicative cells have a limited number of divisions — the 'Hayflick limit' — that we now understand to be caused by telomere shortening; this is sensed by the cells as a persistent DNA damage response². As a consequence, these cells undergo senescence to avoid further genomic instability and accumulation of DNA damage³. In addition to telomere shortening, various other stress stimuli can trigger senescence, including oncogenic stress⁴, oxidative stress⁵, lysosomal or endoplasmic reticulum stress^{6,7}, nutrient depletion⁸, and genotoxic stress induced by cancer therapies⁹ or even from pathogens, including coronavirus^{10,11}. Induction of senescence in response to stress stimuli other than telomere attrition is sometimes referred to as stress-induced premature senescence^{12,13}.

Induction of senescence in response to stress stimuli results in stable cell cycle arrest¹⁴. Therefore, senescence represents a defence mechanism against cancer^{15,16}. However, cell cycle withdrawal is also a feature of several other cell states such as quiescence, terminal differentiation, dormancy or drug-tolerant

persistence¹⁷. It is difficult to unambiguously differentiate between these related cellular states, mainly because we lack 'gold standard' markers of the senescent state. Heterochromatinization of proliferative genes is often seen in senescent cells, and this is thought to enforce a durable cell cycle arrest that cannot be readily reversed by pro-mitogenic stimuli¹⁸. However, as epigenetic modifications are reversible in principle, it is probably incorrect to consider senescence as a strictly irreversible arrest. Indeed, several studies have highlighted the reversible nature of senescence^{18–21}. In addition to resistance to proliferative stimuli, another hallmark of senescent cells is resistance to cell death via resistance to apoptotic stimuli or upregulation of pro-survival pathways. In this way, senescent cells remain in a viable state. Apoptotic resistance is regulated by persistent activation of anti-apoptotic proteins, such as the BCL-2 protein family, as well as epigenetic repression of pro-apoptotic proteins such as BAX^{22,23}. Senescent cells are often also characterized by increased lysosomal capacity¹⁴. This is linked to increased activity of the lysosomal enzyme senescence-associated β -galactosidase (SA- β -gal), which is one of the most widely used markers for senescence although it is neither required nor specific for the senescence phenotype^{24,25}. Other hallmarks of senescence include altered cell metabolism by mitochondrial dysfunction, macromolecular damage, endoplasmic reticulum stress, loss of lamin B1 (a protein of the nuclear lamina), and the overexpression or activation of cell cycle inhibitors INK4A and ARF (both encoded by *CDKN2A*), p21 (encoded by *CDKN1A*) and p53 (encoded by *TP53*)¹².

Arguably, one of the more distinctive, yet not exclusive, features of senescence is the secretion of

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pro-inflammatory cytokines, growth factors and matrix metalloproteinases (MMPs), collectively termed the senescence-associated secretory phenotype (SASP)^{26,27}. The first evidence of a SASP produced by senescent cells was a gene expression study performed in 1999 demonstrating the expression of inflammation-associated genes in senescent fibroblasts²⁸, although the term ‘SASP’ was not used until 2008 (REF.²⁶). The SASP is generated as a result of activation of the nuclear factor κ B (NF- κ B)²⁹, cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING)³⁰, GATA4 (REF.³¹), CCAAT/enhancer-binding protein- β a (CEBP β)³², NOTCH^{33,34}, IL-6 (REF.³⁵), Janus kinase (JAK)–signal transducer and activator of transcription (STAT)³⁶, p38 MAPK³⁷ and mTOR pathways³⁸. The SASP factors can reinforce senescence in an autocrine fashion and influence the tissue microenvironment by paracrine signalling to adjacent tumour, non-tumour and immune cells^{39–41}. Once the levels of senescent cells achieve a certain threshold, they can even produce systemic effects, causing various ageing-related diseases^{4,27,42–44}.

A substantial number of anticancer interventions induce senescence in cancer cells by triggering genotoxic stress, hyperactivation of mitogenic signalling or

oxidative stress, leading to stable cell cycle arrest and SASP induction^{42,45}. Therefore, therapy-induced senescence serves as an initial antitumour mechanism to halt proliferation and prevent further genomic instability^{46,47} (FIG. 1). Through their SASP, senescent cancer cells can also arrest neighbouring cancer cells, improve the vasculature for drug delivery and recruit immune cells that can contribute further to tumour suppression^{29,35,48–53}. However, persistence of therapy-induced senescent cells may be detrimental in the long term by creating a pro-inflammatory, immunosuppressive microenvironment⁵⁴. Moreover, the SASP can promote angiogenesis to advance tumour growth and an epithelial to mesenchymal transition in neighbouring cancer cells, which can enhance migration and promote metastasis^{55–58}. Selective killing of senescent cells — senolytic therapy — has initially been developed to reduce age-related symptoms and improve healthy longevity^{59–62} but senolytic therapy may also have utility for the eradication of senescent cancer cells. Recently, nine Cancer Grand Challenges were identified, one of which was to understand and exploit senescence to improve cancer treatment⁶³. Here, we discuss the opportunities and challenges provided by this ‘one-two punch’ approach for the treatment

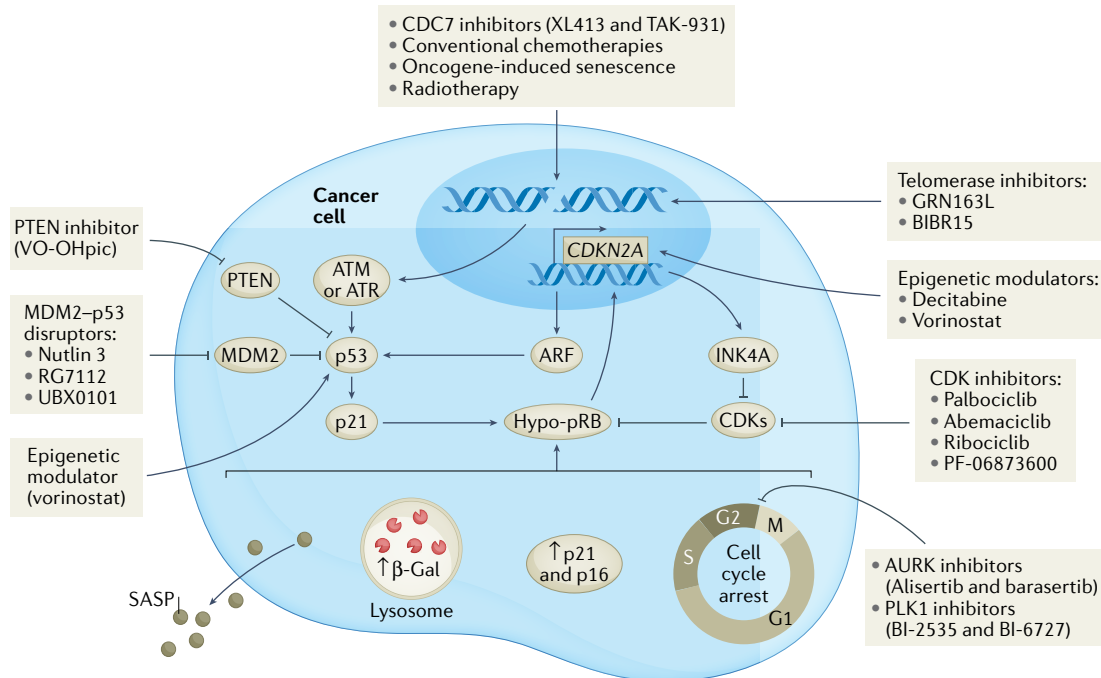


Fig. 1 | Senescence-inducing therapies for cancer. Persistent DNA damage caused by hyperactive oncogenic signals, conventional chemotherapies, radiotherapy, and CDC7 inhibitor (XL413 or TAK931) or telomerase inhibitor (GRN163L or BIBR15) treatment often results in induction of senescence. Massive DNA damage response triggers ATM or ATR signalling and results in p53 and p21 activation. Consequently, cells undergo senescence via hypophosphorylation of RB (hypo-pRB). p53–p21 signalling-mediated senescence can also be triggered by other drugs, such as the PTEN inhibitor VO-OHpi, MDM2–p53 interaction disruptors nutlin 3, RG7112 or UBX0101, or the histone deacetylase inhibitor vorinostat or the DNA methyltransferase inhibitor decitabine can induce CDKN2A expression, which activates both p53-mediated and cyclin-dependent kinase (CDK) inhibition-mediated senescence through its transcripts encoding ARF and INK4A. CDK inhibition-mediated senescence can be induced by inhibitors of CDK4 and CDK6 (CDK4/6) such as palbociclib, abemaciclib or ribociclib. Co-inhibiting CDK2 with CDK4/6 using CDK2/4/6 inhibitor PF-06873600 can achieve more potent senescence induction. Aurora kinases (AURK) and PLK1 inhibitors block the G2/M progression of the cell cycle and this can also induce senescence. These senescence-related pathways are interdependent and reinforce each other. Together, they contribute to several senescence phenotypes, including p21 and p16 upregulation, stable cycle arrest, β -galactosidase (β -gal) activation, and production of the senescence-associated secretory phenotype (SASP).

Box 1 | A one-two punch approach for the treatment of cancer

Cancer is often treated by combinations of drugs to forestall resistance. Such combination therapies can be highly effective, especially when the drugs in the combination target different vulnerabilities of the cancer cell²⁶². A major limitation of drug combinations is their toxicity, which can reduce effectiveness of the cocktail²⁶³. Theoretically, combination toxicity can be circumvented by using sequential drug treatments in which the first drug induces a major new vulnerability that is exploited by the second drug that targets the acquired vulnerability. For this approach to work, the acquired vulnerability induced by the first drug must be maintained after cessation of exposure to the first drug. Senescence appears particularly suited for such a sequential treatment regimen as it is a stable phenotype that can be induced by a variety of stresses²⁶⁴. Moreover, the physiology of the senescent cell is very distinct in terms of metabolism, secretome, transcription and epigenetic states, all of which may represent targetable vulnerabilities. We refer to a sequential treatment regimen consisting of a senescence-inducing drug followed by a second drug that selectively kills senescent cancer cells (senolytic agent) as a one-two punch approach to cancer therapy^{128,242}.

of cancer consisting of pro-senescence and senolytic therapy (BOX 1).

Induction of senescence in tumours

Oncogene-induced senescence. Cellular senescence serves as a powerful protective mechanism against tumorigenesis¹⁶. The activation of oncogenes, such as *HRAS*¹², triggers a growth arrest, referred to as oncogene-induced senescence (OIS), which was first demonstrated in vivo in 1997 (REFS^{4,64–66}) (FIG. 1). After this, in 2005, the concept of OIS was extended in multiple carcinogenesis models, including lymphomas, prostate cancer, lung adenomas, hyperplastic pituitary gland and melanocytic naevi^{66–70}. Melanocytic naevi induced by oncogenic *BRAF* mutations generally remain senescent for decades to evade progression into melanoma⁶⁸. Likewise, the loss of tumour suppressor genes, such as *Pten*, can also induce senescence in primary prostate epithelium, referred to as PTEN-loss-induced cellular senescence (PICS)⁶⁷. The p53 pathway plays a central role in OIS and PICS⁷¹. In OIS, activation of oncogenes leads to DNA damage⁷¹; this in turn activates p53, which induces senescence. This is different from PICS, in which p53 is activated via the mTOR pathway in the absence of clear DNA damage⁷². In addition, OIS can also be mediated by activation of the INK4A–RB pathway, independent of p53 activation and DNA damage signalling^{4,64,73}. Moreover, senescence can also be triggered by other oncogenic pathways such as activated MYC, which increases levels of the ARF-encoding transcript from the *CDKN2A* locus, resulting in stabilized p53 (REF.⁷⁴), and hyperactivated WNT– β -catenin signalling, which triggers a DNA damage response via the p53–p21 pathway⁷⁵. Dysregulation of these pathways by acquired genetic alterations is a frequent event during tumorigenesis as it enables cells to evade a senescence response^{76–80}.

Chemotherapies and radiotherapies. Despite the ability of malignant tumours to evade senescence, they can still be forced to enter a senescent state using therapeutics leading to therapy-induced senescence^{16,81}. Conventional anticancer therapeutics, such as chemotherapy or radiotherapy, are known to induce senescence in cancer cells^{81–84}.

Low doses of chemotherapy particularly trigger a senescent cell state in human cancer cells, while apoptosis is induced at higher doses^{85–87}. This finding might explain why often only a subset of cancer cells become senescent in response to conventional chemotherapies or radiotherapies as the senescence response is only triggered in a specific window of DNA damage. Mechanistically, many chemotherapies cause DNA damage in cancer cells, which triggers senescence through ATM–CHK2 and ATR–CHK1 kinase-mediated activation of the interconnected p53–RB pathways^{88,89} (FIG. 1). Topoisomerase I and II inhibitors, such as doxorubicin, etoposide and camptothecin, are widely used for the treatment of a variety of cancer types and have been shown to dysregulate re-ligation of DNA strands after supercoil unwinding. This leads to massive DNA damage and increased expression of p53 and its downstream targets *CDKN1A* and *PAIL* (also known as *SERPINE1*), subsequently inducing senescence^{90–92}. Platinum-based compounds, such as cisplatin, carboplatin and oxaliplatin, also induce extensive DNA damage through DNA cross-linking, resulting in senescence induction^{93,94}. Similarly, alkylating agents, such as temozolomide, dacarbazine and busulfan, form DNA crosslinks by reacting with atoms in DNA, triggering a DNA damage-mediated senescence response⁹⁵. Microtubule inhibitors, such as paclitaxel, docetaxel and vinca alkaloids, dysregulate the normal microtubule spindle dynamics to impair metaphase–anaphase transition and arrest the cells at mitosis. This cell cycle dysfunction may also cause extensive DNA damage and trigger a p53–p21-facilitated senescence response^{96,97}. Methotrexate and gemcitabine both induce genotoxic stress by blocking DNA synthesis, thereby inducing cellular senescence^{98,99}. It is important to keep in mind that, while quite a few existing chemotherapeutics have some ability to induce senescence, the apoptotic response is dominant in most cancers¹⁰⁰. As such, most chemotherapies are unable to induce senescence in a significant fraction of cancer cells in vivo⁸¹.

Radiotherapy is applied broadly for the treatment of multiple cancer types. This anticancer treatment can induce an irreparable DNA damage response that activates ATM or ATR and p53–p21 pathway-mediated apoptosis and cellular senescence^{89,101,102} (FIG. 1). As, unlike chemotherapy, the treatment can be applied locally, there is less collateral damage to normal tissues and, consequently, potentially also less secondary cancer¹⁰³. Nevertheless, the tissue surrounding the cancer can show an increase in senescent cell burden, resulting in an array of local side effects, including immunosuppressive effects^{27,55,104}.

Cell cycle inhibition. A hallmark of senescent cells is the upregulation of cyclin-dependent kinase (CDK) inhibitor proteins, such as INK4A and p21, to induce cell cycle arrest. By contrast, cancer cells often have upregulated levels of CDKs for progression through the cell cycle¹⁰⁵. Therefore, drugs that inhibit CDKs or enhance levels of CDK inhibitor proteins are currently being investigated for use in senescence-inducing cancer therapy¹⁰⁶. In particular, CDK4 and CDK6 (hereafter referred to

as CDK4/6) are important for progression from G1 to S phase of the cell cycle and are overexpressed in a number of human cancers^{106,107}. CDK4/6 inhibitors mimic the function of INK4A and are able to induce senescence in various cancer cells^{108–113} (FIG. 1). Three CDK4/6 inhibitors — palbociclib, abemaciclib and ribociclib — are approved by the FDA for the treatment of advanced breast cancer. It is important to point out, however, that approval was based on tumour control and not specifically on an ability to induce senescence¹².

CDK2 is another potential therapeutic target for pro-senescence therapy. CDK2 can promote the bypass of OIS and result in tumour progression¹¹⁴. The majority of clinically relevant resistance to CDK4/6 inhibitors, such as loss of *RB1* (which encodes RB) and amplification of *CCNE1* (which encodes cyclin E1) or *CDK6*, could be overcome by CDK2 inhibition to induce senescence^{115–117}. As such, CDK2 depletion was shown to re-sensitize CDK4/6 inhibitor-resistant cells^{117–120}. Unfortunately, the lack of a specific CDK2 inhibitor has, until now, prevented exploiting the synergy between CDK2 inhibition and CDK4/6 inhibition. A triple CDK2/4/6 inhibitor (PF-06873600) for which clinical development is ongoing in breast cancer in combination with endocrine therapy, is a potent senescence inducer in various cancer models^{119,121} (FIG. 1). Unfortunately, the compound also has an affinity for CDK1, which might lead to toxicity as seen with other multi-CDK inhibitors¹¹⁹.

Aurora kinases (AURKs) and polo-like kinases (PLKs) are serine/threonine kinases that regulate several mitotic processes and are potential targets for senescence-inducing therapy. In particular, PLK1 and AURKA and AURKB (also referred to as the AURK–PLK1 axis) are frequently overexpressed in cancer and tightly regulated^{122–124}. Multiple PLK1 inhibitors, such as BI-2535 and BI-6727, and AURK inhibitors, such as alisertib or barasertib, are being tested as potent inducers of senescence in various tumour models in vitro and in mice, and are also currently being investigated in clinical trials^{125–133} (FIG. 1).

Finally, it was shown that inhibition of DNA replication through small molecule inhibition of the kinase CDC7 using XL413 or TAK-931 leads to senescence induction in liver cancer. The senescence response was seen only in *TP53*-mutant tumours, presumably because *TP53*-wild-type tumours retain the ability to mount cell cycle arrest upon CDC7 inhibition, whereas *TP53*-mutant cells will instead undergo senescence as a result of replication stress due to inhibited DNA replication origin firing¹³⁴.

Telomerase inhibition. As mentioned above, replicative senescence occurs in response to telomere attrition. Cancer cells circumvent this most commonly by reactivating telomerase activity¹³⁵. To date, numerous compounds that inhibit the telomerase complex have been identified as candidates for anticancer therapy¹³⁶. Among these, BIBR15 and GRN163L are potent telomerase inhibitors that effectively induce senescence and suppress cancer cell proliferation^{137,138} (FIG. 1). However, the use of GRN163L for pro-senescence therapy should be examined further as this compound also induced apoptosis in human pancreatic cancer cells¹³⁸.

Epigenetic modulators. Another approach to induce senescence is by modulating the epigenome of cancer cells. Decitabine inhibits DNA methyltransferases causing demethylation of the CpG-enriched regions of the *CDKN2A* promoter, inducing INK4A and ARF expression and senescence¹³⁹. Vorinostat, a histone deacetylase inhibitor, can also upregulate the expression of multiple tumour suppressor genes, such as *CDKN2A* and *TP53*, and can induce senescence via these two major pathways in various cancer cell lines^{140,141} (FIG. 1).

Targeting tumour suppressors or oncogenes. Initiation and maintenance of cellular senescence are dependent on p53, which is frequently impaired in cancer cells. In mouse models, genetic restoration of *Trp53* results in regression of sarcomas and liver carcinomas by inducing a senescence response, while an apoptotic response was observed for lymphoma regression^{50,142,143}. In addition, senescence induction was accompanied by upregulation of the SASP and recruitment of immune cells to the tumour, suggesting efficient clearance of senescent cancer cells⁵⁰. Currently, several compounds activating p53 are being developed. For example, the MDM2 inhibitors nutlin 3 and RG7112 can interfere with the p53–MDM2 interaction and show promising results in inducing senescence in tumours that retain wild-type *TP53* in human cancer cell line models^{144–146}. Notably, disruption of the p53–MDM2 complex could also induce apoptosis instead of senescence as demonstrated by the senolytic drug UBX0101 or the forkhead box protein O4 (FOXO4)-DRI peptide in non-cancer models, which is discussed in more detail below^{147,148}. Whether this approach will also work for cancer has yet to be investigated (FIG. 1).

Inactivation of PTEN, for example, with the PTEN inhibitor VO-OHPic, has potential for pro-senescence cancer therapy in vitro and in mice^{72,149} (FIG. 1). It was demonstrated in vitro that *PTEN* status is a significant determinant of cell fate in glioma cells after ionizing radiation; *PTEN*-mutant cells underwent premature senescence while cancer cells expressing PTEN underwent apoptosis¹⁵⁰. In addition, the inactivation of *PTEN* resulted in p53-mediated senescence and suppression of tumorigenesis in mice⁶⁷.

Given the frequent mutation of the *TP53* and *RB1* pathways in human cancer and the notion that these pathways are central to the senescence response, one would think that cancer cells are mostly resistant to induction of senescence. However, this is not the case: nearly all cancer cell lines can be rendered senescent in vitro using DNA damaging agents or AURK inhibitors, and this was independent of *RB1* and *TP53* mutation status¹⁵¹. Apparently, while these genes are mediators in the senescence response of cancer cells, they are not essential for senescence induction in cancer.

Effects of senescent cells in tumours

The SASP factors suppress cancer in part by reinforcing the senescent growth arrest and/or by promoting immune surveillance²⁹. Oncogene-induced and therapy-induced senescent cells secrete the inflammatory cytokine IL-1 α , which is a crucial SASP initiator and

Myeloid-derived suppressor cells (MDSCs). Myeloid cells that specifically inhibit killing of cells by natural killer cells and CD8⁺ T cells.

regulator¹⁵². IL-1 α triggers an autocrine inflammatory response through activation of NF- κ B, which leads to the transcription of IL-6 and IL-8 (REF.¹⁵³) (FIG. 2a). Subsequently, these inflammatory cytokines reinforce senescence proliferation arrest through increased production of reactive oxygen species and a sustained DNA damage response, particularly in oncogene-induced senescent cells^{152,153}. Furthermore, IL-1 α also mediates paracrine senescence in neighbouring cells to suppress tumour progression⁴⁰. Moreover, IL-1 α , IL-6 and IL-8 mediate the recruitment of M1-like macrophages, T helper 1 cells and natural killer (NK) cells to the tumour microenvironment (FIG. 2a). These infiltrating immune cells drive the elimination of senescent cancer cells and might also eliminate non-senescent cancer cells through a bystander effect, although this is not yet proven^{51,154}. In addition, immune cells, such as T helper 1 cells, can also trigger senescence in cancer cells through the secretion of inflammatory cytokines¹⁵⁵.

Although the SASP of senescent cancer cells is initially tumour suppressive by reinforcing growth arrest and promoting immune clearance, it is suggested to be mostly detrimental in the long term^{27,156}. Early evidence for this notion was found two decades ago by an in vivo study demonstrating increased proliferation and tumorigenesis of both premalignant and malignant epithelial cells when co-injected with human senescent fibroblasts in mice¹⁵⁷; the SASP was an important contributor to

this effect¹⁵⁷. Another study observed that MMPs secreted by senescent human fibroblasts were of primary importance in promoting tumorigenesis¹⁵⁸. These prominent SASP factors are involved in the processing and degradation of the extracellular matrix, which can promote cancer cell growth and invasion¹⁵⁹. In addition, MMPs also promote the release of many other cytokines and growth factors supporting tumorigenesis such as vascular endothelial growth factor (VEGF), which promotes tumour-driven angiogenesis¹⁶⁰ (FIG. 2b). Furthermore, senescent cells also secrete the chemokine CXCL1, which promotes tumour growth¹⁶¹.

The SASP factors IL-6 and IL-8 are also important mediators of the pro-tumorigenic effects of senescent cells because they create a chronic inflammatory microenvironment that supports cancer growth^{26,162}. In addition, they drive the transcription of genes encoding MMPs and drive epithelial to mesenchymal transition, thereby promoting tumour invasiveness^{163–166} (FIG. 2b). Another pro-tumorigenic effect of senescent cells mediated by IL-6 is the recruitment of myeloid-derived suppressor cells (MDSCs) to the tumour microenvironment⁵⁵. These MDSCs block IL-1 α signalling and therefore antagonize the establishment of senescence in cancer cells¹⁶⁷. Additionally, MDSCs block immune surveillance by inhibiting CD8⁺ T cells (mediated by IL-6 (REF.⁵⁵)) and NK cells (mediated by the chemokine CCL2 (REF.¹⁵⁴)). In this way, the SASP creates an

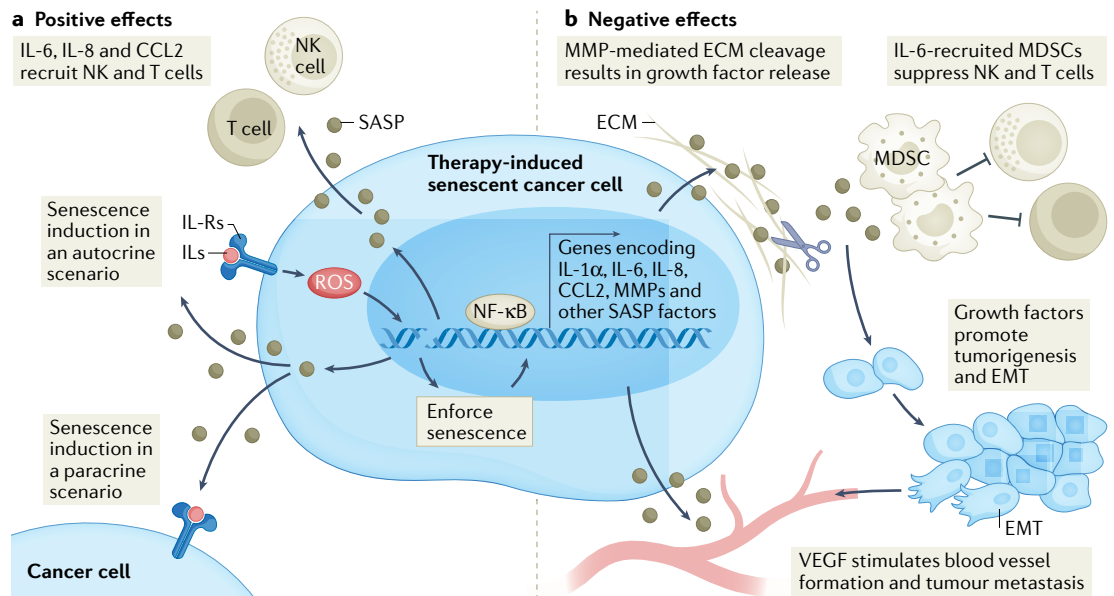


Fig. 2 | **Positive and negative effects of senescent cells in tumours.** Nuclear factor- κ B (NF- κ B) signalling is activated in therapy-induced senescent cancer cells and this drives production of IL-1 α , IL-6, IL-8, CCL2, matrix metalloproteinases (MMPs) and other senescence-associated secretory phenotype (SASP) factors. **a** | The positive effects are that some of these cytokines, such as IL-6, IL-8 and CCL2, can recruit natural killer (NK) cells and T cells contributing to immune surveillance. In addition, IL-6 and IL-8 can reinforce cellular senescence in an autocrine fashion through increased reactive oxygen species (ROS) production and a sustained DNA damage response. These interleukins (ILs) can spread senescence to surrounding cancer cells in a paracrine fashion, which further controls tumour growth. **b** | As the negative effects, IL-6 either secreted by senescent cancer cells or released from the extracellular matrix (ECM) by MMPs recruits myeloid-derived suppressor cells (MDSCs), resulting in an immunosuppressive microenvironment. Moreover, the cleaved ECM components release growth factors that can promote further tumour growth and epithelial-to-mesenchymal transition (EMT), leading to metastasis. Vascular endothelial growth factor (VEGF) from the SASP stimulates blood vessel formation that also contributes to metastasis. IL-Rs, interleukin receptors.

immunosuppressive environment, facilitating tumour growth^{55,168} (FIG. 2b). However, the pro-tumorigenic and anti-tumorigenic effects of senescent cancer cells are likely mediated by a complex interplay between multiple SASP factors and the immune microenvironment. Moreover, the effects of SASP factors will be influenced by tissue type, resident immune cells, inflammatory networks or by the nature of the senescence inducer. As a result, it is presently very difficult to predict whether the effects of senescent cancer cells are pro-tumorigenic or anti-tumorigenic.

Perhaps even more surprisingly, several studies have demonstrated that senescence-inducing therapies are associated with complex reprogramming that could eventually drive stemness in both tumour and normal cells^{21,169}. Moreover, remaining senescent cancer cells that are not cleared by the immune system can spontaneously escape proliferation arrest under certain circumstances and re-enter the cell cycle^{20,21,170}. Another study observed that oncogene-induced senescent cells could also re-enter the cell cycle, particularly by restoring telomerase activity through de-repression of the telomerase reverse transcriptase (*TERT*) gene¹⁷¹. Importantly, senescent cells that resume growth have a WNT-dependent enhanced growth and tumour initiating potential²¹. This senescence-associated stemness results in a highly aggressive tumour, driven by WNT pathway activation independent of the WNT ligand via the SASP and is found to be enriched in relapsed tumours²¹. Moreover, expression of β -catenin in pituitary stem cells provokes a signature of senescence and SASP and can induce craniopharyngioma tumours in a paracrine fashion. Importantly, mice with reduced senescence burden and SASP responses showed decreased tumorigenic potential, indicating that the SASP may drive tumour induction¹⁷². In the past few years, the finding of extracellular vesicles and exosomes as part of the SASP is generating wide interest in the field of senescence. Small extracellular vesicles from senescent cells can also be tumour promoting and reinforce cancer cell proliferation^{173–175}.

Taken together, the role of cellular senescence in tumours and the outcome of senescence-inducing therapies are complex and often unpredictable, mainly because of the dual role of the SASP. The effect of the SASP is highly dependent on context and cell type and variable during the different stages of cancer progression^{27,29,151}. For example, SASP-mediated immunosuppression promotes tumour growth, especially in later stages of tumour progression, while the SASP acts a tumour suppressor in the early stages of tumorigenesis¹⁵⁴. In addition, *TP53* status may be a factor in determining whether the senescence-induced inflammatory response will be tumour suppressive or tumour promoting, respectively¹⁷⁶. In the long term, the SASP of senescent cancer cells is suggested to be primarily detrimental in promoting neoplastic growth, therapy resistance, immunosuppression, metastasis and angiogenesis^{27,55,157,177}. Moreover, senescent cancer cells can potentially remain dormant for a long time, thereby evading therapy and posing a risk for tumour relapse^{16,20,178}. It has also been reported that many genotoxic chemotherapies have debilitating side effects caused by inducing senescence in normal tissues.

The senescent normal cells remain chronically present and promote local and systemic inflammation caused by the SASP, which results in or exacerbates chemotherapy side effects¹⁷⁷. Hence, it could be helpful to combine pro-senescence therapy with senolytic therapy in the context of cancer. Besides direct targeting of the cancer cells by delivering a one-two punch and decreasing the side effects of chemo-radiotherapies on normal tissues, senolytics should eliminate incipient preneoplastic senescent cells, or other senescent cells in the tumour microenvironment, to suppress the detrimental effects of the SASP^{156,162}.

Senolytics for anticancer therapy

As discussed above, persistence of therapy-induced senescent cells after treatment may be detrimental. This argues for a strategy in which therapy-induced senescent cells are eliminated for the long-term good, minimizing risk of tumour progression and avoiding adverse effects¹⁵⁶.

Targeting senescent cancer cells with senolytic agents. One of the hallmarks of senescent cells is a change in chromatin structure that results in changes in gene expression. These alterations can affect fundamental processes like regulation of apoptosis, resulting in acquisition of new vulnerabilities specific to the senescent cells that can be selectively targeted by drugs that could act as senolytic agents^{156,179–181}. Such compounds may have clinical utility in combination with or when used sequentially with senescence-inducing therapies (FIG. 3; TABLE 1).

Senescent cells often have increased levels of the anti-apoptotic BCL-2 family proteins¹⁸². Compounds targeting this family of proteins have been studied intensively for senolytic therapy. Several studies have shown that navitoclax (also known as ABT263) and the related ABT737, selective inhibitors of BCL-2, BCL-XL and BCL-W, effectively eliminated senescent cells of multiple types, including senescent cancer cells, by re-activating the apoptotic pathway^{128,151,183,184} (FIG. 3a). In non-cancer-bearing mice, this was associated with beneficial effects such as rejuvenation and attenuation of age-related diseases^{182,184}. Navitoclax has also shown activity in cancer cell lines and mouse models in combination with various senescence-inducing therapies (TABLE 1), including a class of AURK inhibitors^{128,151}, etoposide^{128,185,186}, doxorubicin¹⁸⁵, olaparib¹⁸⁷ and ionizing radiation^{185,188}, with evidence of reduced side effects¹⁷⁷. Consistent with this, a phase II study showed that rituximab, as a senescence inducer, combined with navitoclax demonstrated higher effectiveness than rituximab monotherapy for patients with chronic lymphocytic leukaemia¹⁸⁹. Furthermore, this combination was well tolerated and resulted in prolonged progression-free survival. As expected, patients with higher levels of BCL-2 responded better to navitoclax treatment¹⁸⁹.

Despite this positive result, several previous clinical studies revealed major side-effects of navitoclax related to on-target effects in haematological cells such as thrombocytopenia and neutropenia¹⁹⁰. Navitoclax has been tested in clinical trials in combination with several conventional chemotherapies. The chemotherapy

Proteolysis-targeting chimaera (PROTAC) drug
A molecule that is capable of removing specific proteins by targeted proteolysis.

doses used in these studies were high enough to induce cell death rather than senescence; possibly as a consequence of this, the outcome of these studies was rather disappointing. Most trials were stopped after phase I due to the lack of efficacy or the high toxicities of the combinations (TABLE 1).

Thrombocytopenia following navitoclax has been shown to result from BCL-XL inhibition; therefore, another BCL-2-selective inhibitor, venetoclax (also known as ABT199), was re-engineered to spare platelets¹⁹¹. However, the senolytic effect of navitoclax relies on BCL-XL inhibition in some cancer types. Consequently, venetoclax is mostly inactive as a senolytic in cancer cells^{128,182}. Other attempts have been made to lower the toxicity of navitoclax. For example, a galacto-conjugated navitoclax prodrug, Nav-Gal, has been recently proposed to enhance the delivery of navitoclax¹⁹². Navitoclax remains inactive in prodrug

form but becomes active when the galacto moiety is cleaved off by SA-β-gal. As senescent cells display high levels of SA-β-gal, navitoclax delivered via Nav-Gal will only be activated by SA-β-gal in senescent cells, thus limiting off-target effects in other cells¹⁹² (FIG. 3b). Moreover, it was also shown in mice that nanocarrier-encapsulated doxorubicin can be activated by SA-β-gal to specifically eliminate palbociclib-induced senescent cancer cells while preventing systemic toxicities¹⁹³. This exciting strategy using nanocarriers targeting senescent cells has been also further validated in other studies^{194,195}. Another approach limiting the platelet toxicity of navitoclax is the use of the proteolysis-targeting chimaera (PROTAC) drug PZ15227, a BCL-XL specific PROTAC that hijacks the cereblon E3 ubiquitin ligase to degrade BCL-XL proteins¹⁹⁶. Cereblon is expressed across human cancer cells but expressed minimally in platelets. As a result, the senolytic specificity of targeting BCL-2 anti-apoptotic

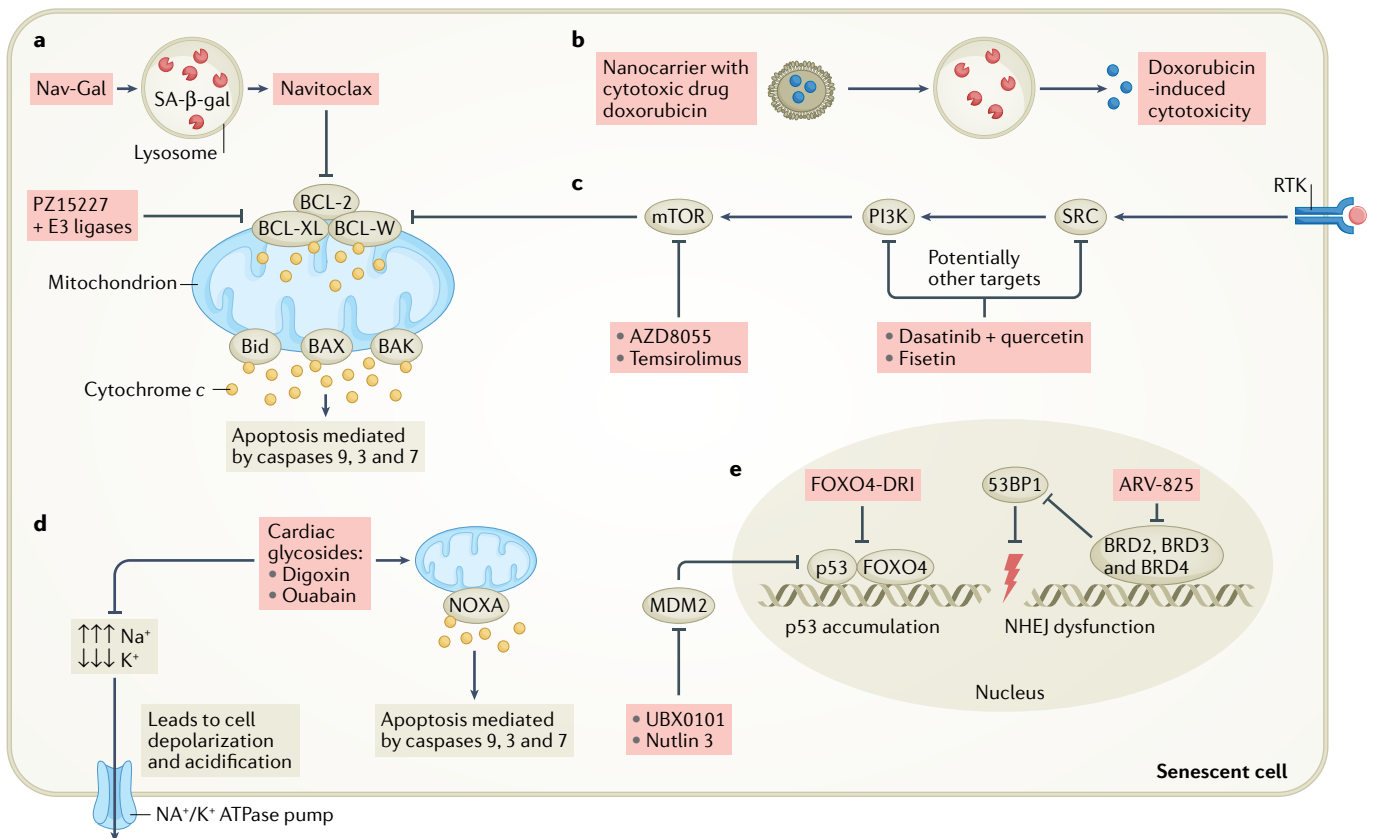


Fig. 3 | Targeting senescent cells with senolytic agents. Changes in senescent cells can induce vulnerabilities that can be targeted by senolytic agents to eliminate these cells. **a** | Anti-apoptotic family proteins are upregulated in senescent cells to confer resistance to apoptosis. Navitoclax, as an anti-apoptotic BCL-2 family inhibitor, can induce intrinsic apoptosis through cytochrome c release that in turn activates apoptosis executioner caspases 9, 3 and 7. Galacto-conjugated navitoclax (Nav-Gal) is processed by senescence-associated β-galactosidase (SA-β-gal) to deploy active navitoclax in senescent cells. PZ15227 hijacks the cereblon E3 ligase to degrade BCL-XL protein. **b** | Doxorubicin can be released by SA-β-gal from encapsulated nanocarriers to induce cytotoxicity in senescent cancer cells. **c** | Dasatinib targets, among others, the SRC kinase and PI3K-AKT signalling nodes. Quercetin or fisetin interfere with the anti-apoptotic protein BCL-XL through inhibition of upstream pathways including PI3K. mTOR inhibition

using AZD8055 or temsirolimus can act downstream of receptor tyrosine kinases (RTKs)-SRC-PI3K and induce senolysis through activation of apoptosis. **d** | Cardiac glycosides, such as digoxin and ouabain, can activate the pro-apoptotic BCL-2 family protein NOXA to induce intrinsic apoptosis. Moreover, cardiac glycosides trigger cell depolarization and acidification through inhibition of Na⁺/K⁺ pumps. Both mechanisms can promote senolysis. **e** | UBX0101 or nutlin 3 can lead to p53 accumulation by disrupting the ubiquitin degradation mechanism mediated by MDM2. The forkhead box protein O4 (FOXO4)-DRI peptide interferes with the binding of FOXO4 to p53, which occurs in senescent cells, and this activates senolysis. The proteolysis-targeting chimaera drug ARV-825 degrades bromodomain proteins (BRD2, BRD3 and BRD4), which disrupts BCL-XL recruitment and interferes with non-homologous end joining (NHEJ), resulting in massive DNA damage-mediated senolysis.

Table 1 | Applications of senolytic treatments in cancer

Agents	Targets or drug mechanisms	Preclinical evidence	Clinical studies ^a
ABT737	Inhibits BCL-2, BCL-XL and BCL-W	With ionizing radiation in breast cancer cell lines and xenografts ¹⁸⁸ ; with etoposide chemotherapy in breast cancer cell lines ¹⁸⁶	NA
Navitoclax (ABT263)	Inhibits BCL-2, BCL-XL and BCL-W	With rituximab (CD20 antibody) in B cell CLL cell lines ¹⁸³	Phase II with rituximab in B cell CLL (NCT01087151) ¹⁸³
		With chemotherapy: etoposide or doxorubicin in breast and lung cancer cell lines and xenograft models ^{128,151,180,185} ; with ionizing radiation in breast and lung cancer lines ¹⁸⁵	Phase I with various chemotherapies: etoposide in small cell lung cancer (NCT00878449) ²⁰² ; irinotecan (NCT01009073) or etoposide and cisplatin (NCT00878449) in various cancers ²⁰² ; paclitaxel (NCT00891605) ²⁰² , docetaxel (NCT00888108) ²⁵⁶ or gemcitabine (NCT00887757) ²⁵⁷ in solid tumours
		With olaparib (a PARP inhibitor) in lung, ovarian, and breast cancer lines and xenograft models ¹⁸⁷	NA
		With AURK inhibitors barasertib or alisertib, or CDK4/6 inhibitor palbociclib in lung, breast, melanoma, colon and liver cancer cell lines ^{128,151,192}	NA
Nav-Gal	Inhibits BCL-2, BCL-XL and BCL-W	With palbociclib in melanoma and breast cancer cell lines; with doxorubicin and ionizing radiation in lung and colon cancer cell lines; with cisplatin in lung cancer cell line and xenograft models ¹⁹²	NA
Nanocarrier-encapsulated doxorubicin	DNA damage	With palbociclib in melanoma and lung cancer cell lines and xenografts ¹⁹³	NA
ARV825	Degrades BET family proteins	With doxorubicin in colon cancer lines and xenografts ¹⁹⁸	NA
Digoxin	Inhibits Na ⁺ /K ⁺ pumps	With alisertib, barasertib, tozasertib (an AURK inhibitor), palbociclib or etoposide in lung, liver, melanoma, breast and colon cancer cell lines ²¹⁰ ; with bleomycin chemotherapy in a lung cancer cell line ²¹¹ ; with gemcitabine in lung cancer xenografts and doxorubicin in breast cancer PDX models ²¹¹	NA
Ouabain	Inhibits Na ⁺ /K ⁺ pumps	With alisertib, barasertib, tozasertib, palbociclib or etoposide in lung, liver, melanoma, breast and colon cancer cell lines ²¹⁰	NA
Temsirolimus	Inhibits mTOR	With docetaxel in prostate cancer cell lines and xenografts; with 5-fluorouracil chemotherapy in breast cancer lines and xenografts ²¹⁴	Phase II with rituximab in diffuse large B cell lymphoma (NCT01653067) ²⁵⁹ Phase I with capecitabine chemotherapy in advanced solid tumours (NCT01050985) ²⁶⁰
AZD8055	Inhibits mTOR	With XL413 or TAK931 (CDC7 inhibitors) in liver cancer cell lines; with XL413 in liver cancer xenografts and <i>Myc^{OE}; Trp53^{-/-}</i> HTVI liver cancer mouse model ¹³⁴	NA
PD1 or PDL1 blocking antibodies	T cell immune responses	With palbociclib + trametinib treatment in <i>KRAS^{G12D}; Trp53^{-/-}</i> pancreatic cancer GEMM and syngeneic pancreatic cancer mouse model ^{49,223} ; with abemaciclib (CDK4/6 inhibitor) in <i>MMTV-rtTA/tetO-HER2</i> breast cancer GEMM ²¹⁹ ; with trilaciclib (CDK4/6 inhibitor) and palbociclib in <i>KRAS^{G12D}; Trp53^{-/-}</i> lung cancer GEMM and syngeneic colon cancer mouse models ²²¹ ; in syngeneic melanoma mouse models with genetic ablation of <i>Cdkn1a</i> and <i>Cdkn2a</i> ²²⁴	Phase II with palbociclib and avelumab (anti-PDL1) in breast cancer (NCT04360941) ²⁶¹ ; many ongoing trials combine PD1 or PDL1 antibodies with chemotherapy but unclear if this involves induction of senescence
CAR T cells	T cell immune responses	With palbociclib + trametinib-induced senescence in <i>KRAS^{G12D}; Trp53^{-/-}</i> lung cancer GEMM ²³³	NA

AURK, Aurora kinase; BET, bromodomain and extraterminal domain; CAR, chimeric antigen receptor; CDK, cyclin-dependent kinase; CLL, chronic lymphocytic leukaemia; GEMM, genetically engineered mouse model; HTVI; hydrodynamic tail vein injection; PDX, patient-derived xenograft; MMTV, mouse mammary tumour virus; NA, not available; Nav-Gal, galacto-conjugated navitoclax; OE, overexpression; PARP, poly(ADP-ribose) polymerase. ^aClinical trials can be accessed using ClinicalTrials.gov.

proteins in senescent cancer cells is increased and the undesired toxicities, such as thrombocytopenia, are reduced (FIG. 3a).

In addition, the senolytic activity of navitoclax is cell type-dependent as upregulation of BCL-2 or other anti-apoptotic BCL-2 family proteins is not a universal senescence vulnerability^{151,179}. The senolytic response to navitoclax is highly divergent among cancer cell lines *in vitro*, and biomarkers of response remain elusive other than the insensitivity of cells with mutations in genes encoding pro-apoptotic BCL2-family members, such as BAX and BAK, to navitoclax treatment¹⁵¹. Thus, loss-of-function mutations in the genes encoding intrinsic pro-apoptotic BCL-2-family members might be a common resistance mechanism for the navitoclax-class of senolytic agents.

Another example of a PROTAC senolytic drug is the bromodomain and extraterminal domain (BET) family degrader ARV825, which is composed of a potent inhibitor of BET proteins BRD2, BRD3, and BRD4 and the E3 ligase binder pomalidomide^{197,198}. ARV825 provokes senolysis through two independent pathways. ARV825 downregulates *XRCC4* gene expression and blocks the recruitment of p53-binding protein 1 (53BP1), which interferes with non-homologous end-joining DNA double-strand break repair and activates apoptosis^{198,199} (FIG. 3e). Moreover, ARV825 also induces autophagy, which may contribute to senolysis¹⁹⁸.

Increased expression of pro-survival and apoptosis resistance networks, including ephrins, SRC, PI3K, p21, BCL-XL and PAI2, was seen in senescent cells¹⁸³. This study also showed that dasatinib, a multi-tyrosine kinase inhibitor that targets, amongst others, the SRC kinase and PI3K–AKT signalling nodes, can kill senescent cells. Quercetin, a natural flavonoid, also has senolytic activity^{183,200,201}. It is not clear how exactly quercetin induces senolysis, but it has been reported that it interferes with the anti-apoptotic protein BCL-XL through inhibition of upstream pathways including PI3K. Administration of a combination of dasatinib and quercetin to aged mice eliminated senescent cells and improved cardiovascular function and survival²⁰². The combination of dasatinib and quercetin as the first-in-human senolytic therapy showed efficacy in treating idiopathic pulmonary fibrosis, a fatal disease associated with the appearance of senescent cells in the lungs²⁰³ (FIG. 3c). Fisetin, another natural flavonol with potent senolytic activity, is twice as potent as quercetin^{47,204}. The efficacy of fisetin alone or in combination with other anticancer agents has been studied in a wide range of cancer types. Fisetin treatment has anti-proliferative and pro-apoptotic effects in cancer cells *in vitro* and *in mice* and suppresses inflammation, migration and metastases in mice²⁰⁴. Although dasatinib, quercetin and fisetin are potent senolytic compounds, they act on a wide range of pathways implicated in various biological processes⁴⁷. The lack of mechanistic insight into how these drugs induce senolysis may be an impediment for their clinical use. Moreover, these senolytic therapies have not shown effectiveness in combination with pro-senescence therapies in animal models of liver cancer²⁰⁵. Nevertheless, the combination

of dasatinib plus quercetin has shown initial promise in the treatment of patients with idiopathic pulmonary fibrosis²⁰³. These drugs are currently also being tested in age-related diseases and in infectious diseases that cause senescence-like phenotypes (TABLE 1).

Compounds that modulate p53 activity can also act as senolytics. For example, the peptide FOXO4-DRI interferes with the interaction between p53 and FOXO4, resulting in p53 nuclear exclusion followed by apoptosis selectively in senescent non-cancer cells¹⁴⁸ (FIG. 3e). In mice, the FOXO4-DRI peptide was well tolerated, improved health span and neutralized doxorubicin-induced chemotoxicity caused by therapy-induced senescent cell burden¹⁴⁸. Although this peptide demonstrates promising senolytic activity in mice, its therapeutic use still needs to be clinically evaluated. The senolytic compound UBX0101 also modulates p53 activity. UBX0101 selectively kills senescent cells by disrupting the interaction between p53 and MDM2¹⁴⁷ (FIG. 3e). A limitation of drugs targeting the p53–MDM2 interaction is that they are not specific for cancer cells or senescent cells, and may therefore cause side effects in normal cells²⁰⁶. However, this could potentially be limited by local drug administration. For example, intra-articular injection of UBX0101 into arthritic joints effectively cleared senescent cells, reduced the SASP and improved joint function in mice¹⁴⁷. Nevertheless, UBX0101 was discontinued after evaluation of the phase I clinical trial data for lack of efficacy²⁰⁷. Whether drugs like UBX0101 have utility in cancer remains to be determined. However, such local administration is mostly impossible in cancer therapy. It should also be kept in mind that p53-targeted senolytic therapy can only be effective in TP53-wild-type tumours, which excludes approximately 50% of all cancers^{208,209}.

Two independent studies identified the cardiac glycosides digoxin and ouabain as senolytic agents with activity in multiple cancer models^{210,211}. Cardiac glycosides can inhibit Na⁺/K⁺ ATPase pump activity resulting in an imbalanced electrochemical gradient within the cell, causing depolarization and acidification. Senescent cells have a depolarized plasma membrane and higher concentrations of hydrogen ions, making them more vulnerable to cardiac glycosides²¹¹ (FIG. 3d). Cardiac glycosides can also alter the pro-apoptotic BCL-2 family protein NOXA through the inhibition of Na⁺/K⁺ transport, resulting in apoptosis²¹⁰. Digoxin behaves as a senolytic at concentrations close to those observed in the plasma of patients treated for heart failure with this drug (20–33 nM)²¹⁰. However, the narrow dosage window of cardiac glycosides might require further optimization for their clinical use as senolytics.

mTOR is an essential regulator of the SASP and mTOR inhibitors have shown senolytic effects in senescent cancer cells^{38,134,212,213}. Treatment with docetaxel and the mTOR inhibitor temsirolimus suppressed prostate and breast cancer growth in mice²¹⁴. Moreover, the mTOR inhibitor AZD8055 acted as a potent senolytic agent for liver cancer cells rendered senescent through CDC7 inhibition (FIGS 1, 3c). This drug combination has been validated in mice by showing its synergistic activity in controlling liver cancer¹³⁴. However, these studies

Immune checkpoint

An immune response that prevents the immune system from attacking cells.

were limited to specific tumour types and the senolytic application of mTOR inhibition remains to be tested in other cancers.

Immune response-mediated senolysis. SASP cytokines, chemokines and other factors that modulate immune cells can either promote or inhibit senescent cell clearance^{29,215} (FIG. 4; TABLE 1). Early studies of the effect of SASP factors on the immune system acknowledged that several factors promote clearance of senescent cells, indicating that the SASP facilitates the removal of senescent pre-neoplastic cells, thus preserving tissue homeostasis. Evidence to support this comes from studies of liver fibrosis, which is associated with an increased risk of cancer. In a carbon tetrachloride-induced liver fibrosis model, carbon tetrachloride treatment can induce DNA damage and lead to upregulation of a ligand for an NK cell activating receptor, NKG2D; this promotes NK cell-mediated cytotoxicity to eradicate senescent cells^{50,52,216,217}. In the *Nras*^{G12V} oncogene-driven hepatocellular carcinoma mouse model, the restoration of *Trp53* expression promoted senescence in cancer cells associated with CCL2 secretion. This chemokine attracts NK cells and, together with NKG2D upregulation on senescent cells, results in NK cell-mediated senolysis¹⁵⁴ (FIG. 4a). As a therapeutic strategy, the MEK inhibitor trametinib and

the CDK4/6 inhibitor palbociclib have been used to induce senescence in the *KRAS*^{G12D}; *Trp53*^{-/-} lung cancer mouse model. Tumour necrosis factor (TNF) and intercellular adhesion molecule 1 (ICAM1), as part of the SASP, were regulated by NF-κB and produced by the therapy-induced senescent cells, and both served as NK cell-activating molecules, promoting cancer cell eradication⁴⁸. This study suggests that, although chronic inflammation caused by SASP induction can have detrimental effects in some scenarios^{26,27,59}, senescence and the SASP can also favour immune system recognition and cancer cell killing. The timely clearance of senescent cancer cells by NK cells in this model underscores that senescence induction can be beneficial⁴⁸.

To study the possibility that increased immune surveillance through therapy targeting the immune checkpoint PD1 can be senolytic, trametinib and palbociclib were combined to induce senescence in a *KRAS*-mutant pancreatic ductal adenocarcinoma mouse model⁴⁹. Besides direct senolytic effects of anti-PD1 antibody therapy in combination with palbociclib and trametinib, the study also demonstrated an impact of therapy-induced senescence on improving tumour vasculature function through SASP-facilitated vascular remodelling. This response increases blood vessel density and permeability, leading to enhanced uptake and activity of

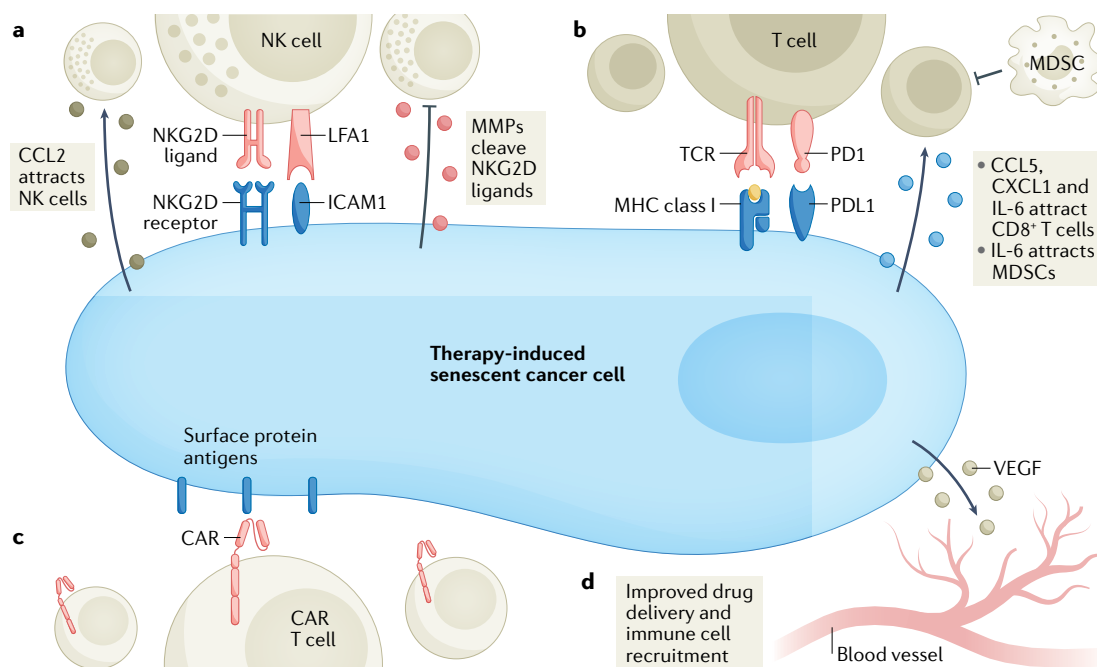


Fig. 4 | Immune response-mediated senolysis. **a** | Senescent cells secrete senescence-associated secretory phenotype (SASP) factors, such as CCL2, and upregulate surface proteins, such as NKG2D and intercellular adhesion molecule 1 (ICAM1), which drive the recruitment of natural killer (NK) cells to promote immune surveillance. However, matrix metalloproteinases (MMPs) may cleave NKG2D ligand expressed on NK cells to dampen the cytotoxic effect of NK cells. **b** | Other SASP factors, such as CCL5, CXCL1 and IL-6, recruit CD8⁺ T cells for senescent cell clearance. Opposite to these immune-stimulatory scenarios, IL-6 can also attract myeloid-derived suppressor cells (MDSCs) to suppress the senolytic effect of NK cells and T cells. Senescent cells may present specific antigens on major histocompatibility complex (MHC) class I to activate CD8⁺ cells. However, as an immune suppressive mechanism, senescent cells can also upregulate PDL1 to attenuate T cell responses. **c** | Chimeric antigen receptor (CAR) T cell therapies armed with chimeric receptors can interact with the surface protein antigens present on senescent cells. Therefore, CAR T cells can specifically recognize and eliminate these senescent cells. **d** | Another SASP factor, vascular endothelial growth factor (VEGF), promotes blood vessel remodelling and can improve drug delivery and immune cell recruitment.

the chemotherapeutic agent gemcitabine, which is the standard-of-care therapy for pancreatic cancer patients (FIG. 4d). Moreover, in the tumour microenvironment, SASP induction facilitates increased endothelial cell expression of VCAM1, a cell surface protein that stimulates lymphocyte adhesion and extravasation into tissues⁴⁹. Other SASP components, such as the pro-angiogenic factor VEGF and the pro-inflammatory cytokines and chemokines CCL5, CXCL1 and IL-6, also facilitate an increase of CD8⁺ T cell infiltration into tumours and improve the efficacy of checkpoint blockade anti-PD1 therapy⁴⁹ (FIG. 4b). These data are intriguing and establish a link between therapy-induced SASP, vascular remodelling and improvement of T cell-based immunotherapies. The previous work of the same group using a mouse *Kras* mutant lung cancer model identified an NK cell surveillance programme without involvement of T cells⁴⁸. Both studies used mouse models driven by the same *KRAS* and *Trp53* mutations and senescence inducers. However, immune surveillance programmes can be somewhat different between tissues. The lungs are generally rich in NK cells but these cells are scarcer in the pancreas²¹⁸, which may explain the different involvement of immune cells²¹⁸. Differences in the SASP produced by different cancer types may further contribute to tissue-specific differences in immune surveillance. Moreover, the effects of pro-senescence therapy on immune responses and anti-PD1 efficacy has been demonstrated in multiple preclinical studies in various cancer types^{219–224} (TABLE 1).

The presence of neo-antigens is a requirement for recognition of any cancer cell by cytotoxic T cells. This begs the question of which neo-antigens are recognized on senescent cancer cells in cases where anti-PD1 therapy shows synergy with pro-senescence therapy. One possibility is that the epigenetic reprogramming that takes place during senescence leads to re-expression of cancer/testis antigens and upregulation of antigen-presentation genes, including those encoding major histocompatibility complex (MHC) class I molecules, which can further enhance T cell recognition of neo-antigens on senescent cancer cells^{49,225}. An interesting other possibility stems from the finding that mRNA transcripts in senescent cells often retain introns²²⁶. Pharmacological perturbation of RNA splicing leads to the generation of neo-antigens that can be recognized by T cells²²⁷. Therefore, senescent cells may create neo-antigens by mis-splicing events. The recruitment of immune cells by the SASP may be synergistic with the expression of such neo-antigens, which could be further stimulated by therapies that enhance T cell function such as anti-PD1 antibodies.

In contrast to the activation of immunity, some studies have reported suppression of immune surveillance by senescent cancer cells. IL-6, as one of the SASP factors, can increase the number of tumour-infiltrating MDSCs, which can suppress the function of lymphocytes and result in a tumour-permissive microenvironment^{29,55} (FIG. 4b). This is similar to the results of other studies, which demonstrated that IL-8 and IL-6 are associated with reduced efficacy of immune checkpoint blockade in patients^{228–230}. In a *PTEN*-null senescent prostate

tumour model, the SASP facilitates the activation of the JAK2–STAT3 pathway through the protein tyrosine phosphatase SHP2 to inhibit immune surveillance by establishing an immunosuppressive SASP-enriched tumour microenvironment that contributes to tumour growth²³¹. Moreover, MMPs in the SASP can cleave the NK activation ligand NKG2D on NK cells to avoid immune surveillance²³² (FIG. 4a).

Together, these studies indicate that combining senescence induction therapies with immunotherapies can potentially improve therapeutic outcomes. Through their SASP, therapy-induced senescence can impact cancer cells and their surrounding tissues by inducing inflammation, recruiting immune cells, spreading senescence to other cells, and altering immune cell function in both positive and negative ways. Additionally, the SASP may vary among senescent cancer cells depending on the tissue type, how senescence was induced, over time, and in response to hormones and, in turn, this may influence the efficacy of immunotherapies^{29,43,215}. To partially overcome these context-dependency issues of immunotherapies, chimeric antigen receptor (CAR) T cell therapies can be developed with chimeric receptors that recognize cell surface proteins upregulated in senescence. This strategy was shown to be feasible with urokinase-type plasminogen activator receptor (uPAR) as a cell surface membrane protein broadly upregulated during senescence; uPAR-specific CAR T cells effectively eliminated senescent cells in vitro and in a lung cancer mouse model²³³ (FIG. 4c). There are other cell surface proteins that are upregulated in senescence that might also be targeted by similar immunotherapies, including NOTCH1 (REF.³⁴), NOTCH3 (REF.²³⁴), DEPI (REF.²³⁵), B₂M²³⁵, DPP4 (REF.²³⁶), NKG2D²³⁷ and ICAM1 (REF.²³⁸). CAR T cell-based senolytic therapies hold promise although cost issues may limit their clinical application²³⁹. Perhaps, antibody–drug conjugates that combine monoclonal antibodies specific to surface antigens with toxins could potentially be a viable application to target senescent cancer cells that show upregulation of certain membrane proteins. Proof of concept for this approach was recently delivered with a B₂M–duocarmycin antibody–drug conjugate, which used a B₂M-targeting antibody linked to duocarmycin as a toxin²⁴⁰.

Future perspectives and challenges

It is generally accepted that combination therapies can forestall resistance development. The concept of combination therapies for cancer has been used clinically since 1958 (REF.²⁴¹). However, toxicity is a major factor limiting the efficacy of drug combinations in oncology. Sequential drug treatment regimens would, at least in principle, allow for a larger array of drugs to be combined as direct combination toxicity is avoided. A one-two punch sequential therapy of pro-senescence therapy followed by senolytic therapy appears feasible in principle as senescence is a stable cellular state that persists after the senescence inducer is withdrawn^{128,242}. As such, pro-senescence therapy is well suited for use in a sequential treatment regimen. Thus, a first unmet need is a lack of drugs that are highly effective in inducing senescence in a high proportion of cancer cells.

Cancer/testis antigens

Antigens expressed primarily in male germ cells and various malignancies.

Chimeric antigen receptor (CAR) T cell

T cells expressing artificial receptors, which can recognize certain proteins on cancer cells.

Drop-out screens

Loss-of-function genetic screens that identify genes whose depletion leads to lethality.

In addition, such drugs should show selectivity for cancer cells over normal cells as inducing senescence in normal tissues (for example, as occurs with many genotoxic chemotherapies) can cause detrimental side effects¹⁷⁷ (FIG. 5).

Another challenge is the lack of gold standard biomarkers of the senescent state. It is evident that no single marker can unambiguously discriminate between senescence and other growth-arrested states. Several laboratories have developed multi-gene signatures to describe senescence in primary cells. For example, we have recently used a panel of senescent cancer cells to identify the SENCAN classifier for cancer senescence¹⁵¹. In vivo, detection of senescent cells could be achieved by using galacto-conjugated fluorescent nanoparticles, which have been tested in models of chemotherapy-induced senescence^{193,243}. To measure efficiency of senescence induction in tumours of patients on therapy, non-invasive imaging modalities would be ideal. One option might be to use a radioactive β -gal PET tracer for this purpose, although this is still in early development^{207,244} (FIG. 5). However, β -gal associated detection strategies might be insufficient to define all senescent cells. Cells from some tissue types do not induce SA- β -gal activity when they become senescent²⁴⁵. Macrophages also tend to exhibit increased SA- β -gal activity^{246,247}; therefore, false positives may result from macrophages inside inflamed tumour tissues. Other non-invasive strategies may also detect clearance of senescent cells. For example, oxylinin biosynthesis is significantly increased during senescence²⁴⁸. In particular, the intracellular prostaglandin dihomogammalinol-15D-PGJ2, one type of oxylinin, specifically accumulates in senescent cells and is released during senolysis. Importantly, dihomogammalinol-15D-PGJ2 could be measured from urine and blood samples from humans²⁴⁸ (FIG. 5). In addition, other markers, such as cleaved uPAR, could potentially be used in non-invasive approaches to detect senescent cancer cells^{233,249}. However, it should be further investigated whether these markers can be used to detect senescent cancer cells in different contexts, such as cells that have diverse genetic backgrounds, arise in different tissue

types or have undergone senescence by different agents. In addition, non-invasive detection of SASP factors in plasma could potentially reveal senescent cell burden²⁵⁰. Non-invasive detection of senescent cells should be invaluable in future clinical trials of pro-senescence and senolytic combinations.

A further issue that needs to be solved is the lack of broadly acting senolytic drugs. Most senolytic drugs were developed with the aim to reverse the effects of ageing and were consequently tested mostly on primary cells. Our recent study of the most commonly used senolytic (navitoclax) on a panel of senescent cancer cells showed that it has widely variable activity as a senolytic¹⁵¹. Thus, apart from the lack of an absolute marker of the senescent state, the field is also in need of a druggable and broadly present vulnerability of senescent cancer cells. We have developed an inducible CRISPR–Cas9-based genetic screening platform in which gene-editing is activated only after senescence is induced in cells. This allows for performance of drop-out screens to identify novel senolytic targets on the genome scale. If universal vulnerabilities of senescent cancer cells exist, unbiased genetic screens should allow their identification.

A further challenge that is certainly not unique to senescence-based therapies is the issue of tumour heterogeneity. Tumour heterogeneity leads to variable drug responses that may limit the effectiveness of senescence induction within tumours. Will it be necessary to provoke a senescence response in most inherently heterogeneous cancer cells, or will senescent cells aid in the killing of non-senescent cancer cells through a bystander effect? Senescent cells can spread the senescent phenotype through the SASP to the surrounding non-senescent cells within tumours²⁵¹, which may sensitize non-senescent cancer cells to senolytic treatments; however, this has not been proven so far. Moreover, such bystander effects could be fortified by the local tumour microenvironment shaped by the SASP of the senescent cells. For instance, it is thought that the clinical efficacy of immunotherapy is, at least in part, caused by the killing of subpopulations of resistant cancer cells through

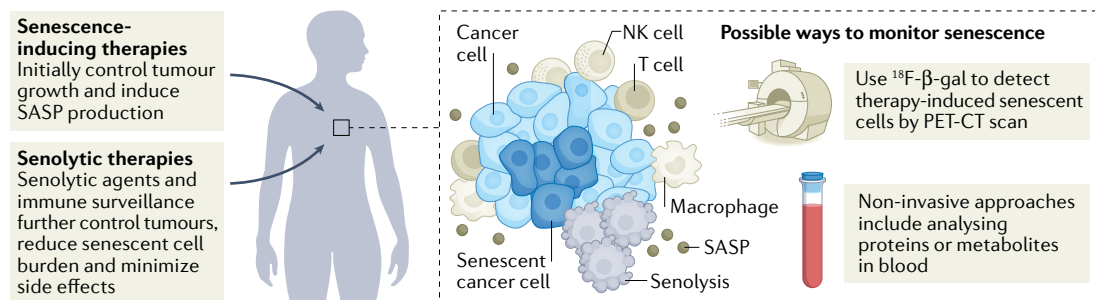


Fig. 5 | Future perspective for senescence-based therapies. To achieve better antitumour responses and to inhibit tumour progression mediated by senescence-associated secretory phenotype (SASP) factors, senescence-inducing therapies can be combined with senolytic treatments. Senolysis can also be mediated by recruitment of immune cells by the SASP factors produced by senescent cells. Detection of senescent cells within the patient's tumour is important to assess the efficacy of pro-senescence therapies. One way this can potentially be achieved is by a PET-CT scan using an ^{18}F -labelled β -galactosidase tracer (^{18}F - β -gal). As potential non-invasive approaches, senescence-associated proteins or metabolites can be detected to measure senescence burden in blood. These technologies provide possibilities to evaluate the efficacy of pro-senescence–senolytic treatment responses and could help guide future clinical trials. NK, natural killer.

Senomorphic drugs

Drugs that modify the senescence-associated secretory phenotype produced by senescent cells.

a bystander effect. While the contribution of bystander effects in the context of senescence-inducing therapies are understood only poorly to date, it may represent an opportunity to overcome tumour heterogeneity.

It will be crucial to better understand how the SASP produced by senescent cancer cells impacts the interaction between senescent cancer cells and the immune system. Some early data point towards a synergy between pro-senescence therapy and checkpoint immunotherapy^{48,49}. It is important to keep in mind that there is a publication bias for experiments that yield positive results. It is therefore possible that not all pro-senescence therapies and not all cancer types will benefit from combination with checkpoint immunotherapy. Such notion is supported by the substantial heterogeneity in SASP factors produced by different senescent cells¹⁵¹. Thus, it will be important to understand when a SASP provokes an immune response that can be enhanced by checkpoint immunotherapy and when it does not. It may become possible to use so called senomorphic drugs such as the NF-κB-inhibiting

drugs apigenin and kaempferol or the mTOR inhibitor rapamycin^{212,252}, which can modify the SASP of senescent cells to become more responsive to checkpoint therapy clearance²⁵³.

Finally, ablation of senescent normal cells by senolytic therapy in aged individuals needs to be carefully evaluated. In the elderly, senescent cells can be a high percentage of the net number of cells in some tissues and this might conceptually jeopardize tissue structural integrity or affect vascular endothelial cells, leading to blood–tissue barrier disorder that may, in turn, lead to liver and perivascular tissue fibrosis and health collapse^{254,255}. This potential issue highlights the need for cancer-selective senolytics to be developed.

Thus, while many questions currently remain unanswered, key advantages of senescence-based therapies, such as sequential treatment opportunities and attraction of immune cells by senescent cells, warrant further investigation of this approach.

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All authors researched data for the article, contributed substantially to discussion of the content, and wrote the article. L.W. and R.B. reviewed and/or edited the manuscript before submission.

Competing interests

R.B. is founder and shareholder of a company that exploits senescence for cancer therapy: Oncosense. R.B. and L.W. are part time employees of Oncosense. L.L. declares no competing interests.

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